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# Pancreatic Cancer Subtypes: A Roadmap for Precision Medicine.

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## Abstract

Pancreatic ductal adenocarcinoma (PDAC) is projected to become the second cause of cancer-related deaths by 2020. Although it has traditionally been approached as one disease, accumulated evidences point to the clinical heterogeneity of this disease which translates into disparity in outcomes between patients. Much emphasis has been put into patient classification introducing a platform for more tailored therapies. In the last ten years, there have been important advances in the understanding of the molecular pathogenesis of PDAC, which has culminated with a comprehensive integrated genomic analysis from RNA expression profiles. Bailey *et al*. defined four subtypes and the different transcriptional networks underlying them. We will first briefly describe and compare different subtyping approaches which are mostly based on tissue mRNA expression analysis. We will propose that these latest approaches to disease classification embrace not only those patients that are surgically resectable (20%) but even patients ineligible for surgery. Such considerations will include possible reclassification of these specific subtypes, enabling more personalized diagnosis and individualized treatment. The ultimate goal of this review is to *identify* current challenges in this area and *summarize current* efforts in developing clinical modalities that can effectively identify these subtypes in order to advance Precision Medicine.

### Keywords

Pancreatic cancer; subtype classification; precision medicine; genomic analysis; liquid biopsy

## Introduction

Progress in survival among patients with solid tumors can be credited to effective modalities of early detection and identification of cancer subtypes. Though the former has been somewhat evasive for pancreatic ductal adenocarcinoma cancer (PDAC), subtyping of this tumor has developed over the past 5 years, culminating in a Nature publication last year that provided genetic signatures for four basic molecular subtypes (1). Classifying altered levels of gene expression led to the establishment of gene programs (GPs) that are involved in similar cell signals or functional unit. Allowing groups of genes to be considered as a single entity and employed to define a molecular profile of each subtype was pivotal to this work.

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Indeed, these subtypes were shown to have similar yet distinct genetic and epigenetic signatures (eg. DNA methylation), which are well-correlated with survival not only between subtypes but even among GPs from each subtype. In addition, a comparison with cell lines derived from KPC mice, a highly characterized and frequently employed mouse model of PDAC, was included.

Considering Bailey's classification as a reference, we will revise other types of classifications established before, giving brief description of these works and comparing the molecular features used to differentiate each subtype. A detailed mutational landscape of these pancreatic cancer subtypes is beyond the scope of this review and has been elegantly summarized recently (2,3). However, the utility of these subtypes and their potential extension in both clinical and research settings will be addressed. Considering that the majority of subtype classifications are based exclusively on about 20% of PDAC patients (which represents only patients with early stage disease eligible for surgery), we will also comment on other approaches employing serum as a source of information that could be applied to any PDAC patient, regardless of stage. Future work will be considered including correlations of all these subtypes with histopathology, *in vivo* models of PDAC, and potential responses to current and future therapeutic regimens, a strategy previously utilized to identify the first set of PDAC subtypes (4). The ultimate goal of this review is to revise current efforts in developing modalities in the clinic that can effectively identify these subtypes in order to advance Precision Medicine.

## Large-scale genomic analyses based subtypes:

Previous reports identified specific signaling pathways that are commonly observed in PDAC, some of which are likely components involved in its etiology(5). Employing such findings, Bailey *et al* selected signaling cascades (*KRAS*, *TGFB*, *WNT*, *NOTCH*) and/or cellular functions (cell cycle, DNA repair, RNA processing) to define various genes programs (GPs), and collected data supported this initial supposition. Initially using a small cohort of 96 tumors for clustering of RNASeq data, four groups readily emerged from which PDAC subtypes were defined. Many of these had similarities with previously published PDAC subtypes ((4,6) Table 1), which served to confirm this approach and the data collected. This approach was then expanded using an additional cohort of 343 patients.

Whole genome and deep-exome sequencing of 456 PDAC samples collected after surgical resection were completed and utilized to identify a comprehensive genetic mutational analysis or landscape. From this work, ten molecular systems captured these mutated genes including *KRAS*, G1/S checkpoint, *TGFβ* signaling, histone modification, and others. Importantly, these 10 molecular characteristics were overlapping between the PDAC patients. Also, 50 regions of gain (7 chromosomal arms, generally oncogenes) and 73 regions of loss (12 chromosomal arms, typically tumor suppressor genes) were identified in this cohort of PDAC patients. RNASeq was the second evaluation performed on these samples to confirm, in part, findings from DNA exome sequencing and extend this approach to identify transcriptional networks that could define specific subtypes. Of 26 defined networks and downstream targets related to development and regeneration, 10 GP were used to discriminate 4 PDAC subtypes: (1) squamous; (2) pancreatic progenitor; (3)

immunogenic; and (4) aberrantly differentiated endocrine exocrine (ADEX). These data were assembled into heat maps with altered gene expression (constitutive mutant, partial or complete loss, etc.) selected for specific categories of genes based on their commonality among a given pathway. These heat maps could be readily organized into GPs which were used to identify individual PDAC subtypes based on an empirically established algorithm.

### Molecular Features of PDAC Subtypes

The *Squamous* Subtype (31% incidence) has the largest and most diverse set of GPs (GP2–5) than any other subtype and is correlated with significantly worse disease outcomes (13.3 months), with nearly half the survival rate compared to the average of the other three subtypes. This is likely due to the sheer number and variations of systems that are impacted in this subtype including altered transcriptional activity (p63), inflammation, hypoxia, metabolism (likely including oxidative stress), *ECM*, *TGFβ* and *WNT* signaling cascades, activated *MYC*, and autophagy.

The *Pancreatic Progenitor* Subtype (19% incidence) is characterized by a single GP (GP1) comprised of developmental transcription factors including *PDX1, HNFS, FOXAS, HES1*, and metabolic features including those linked to fatty acid oxidation, steroid hormone and drug metabolism, and glycosylation. The latter has downstream impact on mucins, implicating a greater presence of IPMN which is observed in this subtype. Some patients with this subtype survive 4 years or longer though overall outcomes (23.7 months) are marginally less than the Immunogenic (25.6 months) and ADEX (30.0 months) subtypes.

The *Immunogenic* Subtype (29% incidence) captures the same GP as that for the Pancreatic Progenitor Subtype (PG1) but has an additional three GPs (GPS 6–8) that contain a family of genes related to immune cell function including aberrations related to B-cell function, antigen presentation, $CD4^+$ and $CD8^+$ T cell signaling, Toll-like receptor (*TLR*) signaling, and upregulation of *CTLA4* and *PD1*. For this particular subset of patients, the underlying rationale would be that restoration of critical immune system functions in addition to checkpoint inhibition could offer some benefit and improve outcomes for these patients.

The *Aberrantly Differentiated Endocrine Exocrine (ADEX)* Subtype (21% incidence) includes two GPs (GPs 9–10) with one focused on exocrine function/secretion (*MIST1, NR5A2*, etc.) and the other related to genes involved in β-cell development (*MODY*, *INS*, *NEUROD1*, *NKX2–2*, etc.) and those upregulated following mutant *KRAS* expression. The likely culmination of endocrine and exocrine dysfunction may represent a causative link between pancreatic cancer and diabetes.

## Comparison between PDAC classifications

Sequencing of the human genome has profoundly altered our understanding of the biology and diversity of this disease. The birth of personal genomes and genomic medicine has been made possible by extraordinary advancements in sequencing technologies over the past 10 years(7). Global genomic analyses were applied to human pancreatic cancer patients in the search of new insights into the tumor pathogenesis. In 2008 Jones *et al*. performed a comprehensive genetic analysis of 24 pancreatic cancers and determined 69 gene sets that

were genetically altered in the majority of the 24 cancers examined, defining a core set of 12 cellular signaling pathways and providing data required for personalized cancer medicine(5). This research opened the search of new therapeutic options for patients with pancreatic cancer. Although these results point to the utility of personalized medicine, the works by Collisson *et al.*(4) and Moffitt *et al.* (6) set the stage for categorizing PDAC into classifications or subtypes. Both were pivotal in providing a platform for grouping pancreatic cancer as a means to improve patient stratification for more tailored therapies, the goal of Precision Medicine. In this capacity, both reports serve as foundation for the latest subtyping described in Bailey *et al.*(1).

In 2011, Collisson *et al.*(4) applied global gene expression analysis for subtype identification. In order to overcome the limitation of tumor specimens available for this study, the authors combined analysis of transcriptional profiles of primary PDAC samples from several studies along with human and mouse PDAC cell lines. They merged one dataset generated by their group using 27 microdissected PDAC material and another previously published dataset(8). From the combination of these two clinical datasets, they proposed a 62-gene signature designated as *PDAssigner*, that could classify subtypes as classical, quasi-mesenchymal (QMPDA) and exocrine-like based on specific gene expression. As a further validation process, they applied the *PDAssigner* to three additional published PDAC expression datasets(9–11) and were able to classify these samples in the aforementioned three subtypes. Moreover, *PDAssigner* was a predictor of overall survival. They also attempted to apply the classifier to a collection of 19 human and 15 mouse PDAC cell lines but were not able to find any representation of the exocrine-like subtype. Finally, they proposed subtype-specific drug responses to Gemcitabine and Erlotinib using cell lines. QM-PDA subtype lines were, on average, more sensitive to Gemcitabine than the classical subtype. Conversely, Erlotinib was more effective in classical subtype cell lines. Using a recently established patient-derived PDAC cell line (Pancreatic Ductal AdenoCarcinOma or PACO), the three subtypes defined by Collison *et al.* have been described (12). Two markers, *HNF1A* and *KRT81*, were identified by immunohistochemistry to stratify tumors into the three subtypes. Individuals with resectable *HNF1A*+ exocrine-like PDAC were found to have the best survival rates. In 2015, Moffitt *et al.*(6) extended on the work from Collisson *et al.* by defining two tumor subtypes while adding stromal classifications. One of the tumor subtypes is classified by a more universal tumor identity. The basal-like subtype defined was consistent with other cancer basal-like subtypes such as bladder or breast and is comprised of tissue with high content of laminins and keratins. The Moffitt classical subtype overlapped with Collison's classical subtype and is characterized by high adhesion-associated proteins, ribosomal and epithelial gene expression, and elevated *GATA6* expression. When comparing with the other two subtypes described by Collison, Moffitt stated that the QM subtype is partially driven by non-tumor contributions of the stroma and the exocrine-like subtype by the normal pancreas. As stated before in Collison's model, cell lines failed to represent the different subtypes described. Likewise, all cell lines (as well as the majority of the metastatic samples) in Moffitt's were classified as "basal-like", suggesting once again that cell line models represent only one subset of PDAC.

The primary uniqueness of this work is the definition of stromal subtypes (*normal* and *activated*), besides the tumor subtypes as described above, which were distinct in separating

tumor, stroma, and normal gene expression profiles. The work included analyses of gene expression in a cohort of microarray data from 145 primary and 61 metastatic PDAC tumors, 17 cell lines, and adjacent normal samples from 46 pancreatic and 88 distant sites. Validation using RNA sequencing was performed on 15 primary tumors, 37 pancreatic cancer patient-derived xenografts (PDX), 3 cell lines, and 6 cancer associated fibroblast (CAF) lines.

The most recent subtyping classification presented by Bailey *et al*.(1) involves a comprehensive integrated genomic analysis of 456 pancreatic ductal adenocarcinomas to define differential expression of transcription factors and downstream targets. Initially, they enriched the samples, selecting tumors with high epithelial content (≥40%) to balance stromal gene expression. When this transcriptome classification is compared with the other two previously published studies, Bailey and Collison classifications overlapped except for the addition of the immunogenic subtype. The Collison QM corresponds to squamous, classical corresponds to pancreatic progenitor, and exocrine-like to ADEX. On the other hand, there was less similarity between the Moffitt and Bailey subtypes, probably due to the reduced stromal component in the latter. A comparison of these three subtypes has been summarized in Table 1.

In 2015, Waddell *et al*. performed whole-genome sequencing and copy number variation analysis of 100 PDAC samples showing that variation in chromosomal structure is an important mechanism of DNA damage in pancreatic carcinogenesis and employed structural variation profiles to classify PDAC into four subtypes (stable, locally rearranged, scattered and unstable subtypes, according to the structural variation events) with potential clinical relevance to platinum-based therapies(13). Recently, a report linked mutation burden (defined by whole genome and exome sequencing) as a marker for immunotherapy response, in a subset of mismatch repair-deficient patients which represent a small, but meaningful proportion of pancreatic cancers with a prevalence of 1% (14). Another approach for PDAC patient classification combined histopathological and molecular profiles. According to tumor morphology, PDAC was classified as *conventional type*, which showed an equal mixture of various histological elements; *combined PDACs*, which were characterized by a dominant histological component (defined as involving more than 30% of the tumor area) and *PDAC variants* including adenosquamous, colloid, papillary and special carcinomas. Combining these classifications and the mutational status of the four driver genes (*KRAS*, *CDKN2A/P16*, *SMAD4* and *TP53*) correlated with PDAC outcome(15). All these studies show that unlimited number of characteristics can be selected as features to classify patients, which translated into the same number of different classification approaches. We acknowledge the combined approach in (15) where both histopathologic heterogeneity and genetic profile correlate with survival.

Previous genome sequencing efforts have focused on tumors with relatively high neoplastic cellularity (either by using techniques that purify tumor samples, microdissection of the tissue or generating cell lines or patient-derived xenografts). However, it has been demonstrated that tumor purity can influence cancer subtypes. In 2017, Raphael *et al*. performed a multi-platform analysis (genomic, transcriptomic and proteomic profiling) of 150 pancreatic cancers accounting for neoplastic cellularity highlighting that depth of

sequencing is important for the detection of mutations and somatic copy number alterations in low-purity samples(16). Two tumor-specific subtypes of PDAC were proposed: basal-like/squamous (enriched in *TP53* mutations) and classical/pancreatic progenitor (enriched in *GNAS* mutations). These two subtypes also harbored specific regulation of miRNA and DNA methylation. By means of protein expression analysis, they also described prognostic subtypes with a group of tumors displaying improved overall survival which harbor elevated *RTK* and *MTORC* signaling.

## Other approaches for PDAC classification:

Much effort has been focused on identifying specific PDAC subtypes due to the impact that tailored therapies can have in PDAC patient survival. However, the clinical application of PDAC subclassification is lacking due to less than reliable biomarkers that define these molecular subtypes. It has become evident that genetic changes alone are not enough to understand most PDAC. Thus, genomic data is to be complemented with proteomic (and other-omics) based approaches to decipher the active molecules driving PDAC.

Using Immunoaffinity-coupled high-resolution mass spectrometry over two panels of PDAC cell lines, three different subtypes have been described based on distinct tyrosine phosphorylation profiles (signaling networks associated with cell-cell adhesion and epithelial-mesenchyme transition, mRNA metabolism, and receptor tyrosine kinase (*RTK*) signaling)(17). Interestingly *RTK*-enriched cell lines exhibited enhanced sensitivity to the small molecule *EGFR* inhibitor erlotinib, indicating that their phosphosignature may provide a predictive biomarker for response to this targeted therapy. As an example of all the discrepancy that exists regarding classifications methods, the authors of this last paper could not find any correspondence of their classifier with those obtained via transcriptomics or gene mutation patterns. Considering that Moffitt *et al* assigned all the PDAC cell lines to a single subtype (basal), this approach is, according to the authors, clearly resolving subtypes not detected by these works. It should be noted that the fact that we can determine an altered transcriptome in a patient does not mean it is going to be translated and manifested phenotypically. In this sense we consider that the combination of different-omics technologies could give a more detailed profile of changes to classify PDAC patients.

These are examples of elegant works trying to classify PDAC patients. However, the source of information remains primary tumor. The challenge here is being able to diagnose the PDAC subtype just with a blood test and hence, adapt the treatments accordingly. In an attempt to expand the classification to all PDAC patients, regardless of the stage, serum biomarkers arise as an ideal source of molecular signatures. Tumor marker carbohydrate antigen CA19–9 has been widely used in PDAC diagnosis and is still the current standard serum tumor marker. Nevertheless, there are some limitations in the usefulness of this marker including its elevation in only about 65% of individuals with resectable pancreatic cancer, poor utility in screening asymptomatic populations, lack of discernment between pancreatic cancer and chronic pancreatitis, and appearance in many other malignances. In this way, the European Group of Tumor Markers (EGTM) the National academy of Clinical Biochemistry (NACB) recommend that CA19–9 should not be the only indicator used for diagnosing PDAC(18). Several combinations with CA19–9 have been considered, with a

recent report demonstrating the improved specificity and sensitivity of CA19–9 with thrombospondin as a combined approach in asymptomatic individuals who have a high risk of developing PDAC (19). Yet, there remains an urgent need to identify additional serological biomarkers for the detection of early stages of PDAC with full appreciation for the heterogeneity typically observed in these patients. As Harsha *et al* commented in their paper describing an excellent compilation of potential biomarkers of pancreatic cancer(20), the problem with serum biomarkers in PDAC could be that there are too many. The challenge should be focused on validation of previously identified biomarkers and the pursuit of classifications based on them. This is an area of research that still requires greater effort.

Circulating tumor cells (CTCs) could represent another source of blood-based molecular profiles. CTCs are tumor cells shed off from a primary tumor into the circulation and it is widely accepted that they are involved in the metastatic migration of solid tumors to distant sites(21). Thus, the use of CTCs as a real-time liquid biopsy has received attention over the past years(22). Association between CTCs and cancer subtypes have been previously described mainly for breast cancer(23,24), showing also prognostic impact to tumor responses(25). Very recently it has been proposed that CTCs may serve as potential biomarkers for PDAC(26). In this report, the authors conclude that both CTC subtype (triploid, tetraploid or multiploid cells) and total number were upregulated in the peripheral blood of PDAC patients when compared with healthy controls, serving thus as a diagnostic tool for the disease. Another recent study showed that patients with >3 CTC/ml tend to have a worse overall survival (OS) than patients with 0.3–3 CTC/ml(27). However, isolation and enrichment of the samples are very challenging processes, particularly for PDAC, which has been defined as a cancer with one of the lowest number of CTCs in circulation(28). Although we tend to think about genes when dealing with heterogeneity, one cannot ignore other sources of variability including metabolic rewiring. Although the apparent differences in energy metabolism in different tumors are attributable to their intrinsic genetic, epigenetic, and microenvironmental characteristics, they may also represent distinct subtypes(29). In this regard, the following subtypes have been proposed: “glycolytic” PDAs (comparable to the QM-PDAs), with elevated glycolysis and serine pathways, increased monocarboxylate transporter 1 (MCT1) expression, and high glutamine incorporation into TCA cycle metabolites; “lipogenic” PDAs (comparable to classical PDAs), with lipid and electron transport chain metabolite enrichment and high lipogenesis gene expression, high oxygen consumption and mitochondrial content, and high glucose incorporation into TCA cycle metabolites; and “slow proliferating” PDAs low in amino acids and carbohydrates. These subtype-specific cell lines were also shown to have different responses to various metabolism-based inhibitors(30). For example, mesenchymal tumors may be more vulnerable to ROS-inducing agents and antioxidant responses.

## Utility, Limitations and challenges in PDAC Subtyping

PDAC is a heterogeneous and molecularly complex disease with significant differences observed in outcomes among individual patients. Hence, there is a need towards tailored therapies specific for genetic, even biomolecular characteristics of individual tumors, including PDAC and other cancers(31). In clinic, cancer typically is identified by

histopathological analysis or by different, well-established imaging modalities including computer tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), X-ray, and ultrasound (US). Histological analysis is the gold standard for clinical diagnosis of cancers and for contributions to prognosis, identification of disease stage, and potential therapeutic targets(32). Histopathology requires tissue biopsies and subsequent examination by a pathologist(s), which still remains somewhat subjective. In more recent years, a reshuffling of PDAC classifications has been underway, from the histopathologic subtypes (which traditionally define PanIN or cystic-derived PDAC) to the molecular subtypes determined from various high-throughput profiling techniques previously described in this review.

A predominant advantage of PDAC subtyping is related to Precision Medicine. Molecular subtyping provides information that can substantially impact the selection of treatments for this disease particularly at specific stages. For a metastatic disease, such as PDAC, systemic chemotherapy is the most common treatment modality, though its benefits are often marginalized. The traditional “one-size-fits-all” approach can lead to a patient’s unnecessary exposure to the toxic effects of treatments without much of a survival benefit. Patient stratification is a critical component to this approach. Identifying unique therapeutic targets while also exposing novel targets can be tested for clinical use and ultimately establish treatments for each individual subtype. In the search for innovative approaches to treat PDAC, one example would be therapies that specifically target mechanisms of immune evasion (that are currently in clinical trials for other cancer types), which could be tested in patients with an Immunogenic Subtype. Immune modulators could be used in this PDAC subtype and potentially targeted to restore specific mechanisms that will prompt tumor cell death by the immune system. Hence. testing in clinical trials is encouraged. Indeed, the genetic, stromal, and immunologic features of PDAC have been interrogated defining immunologic subtypes. The mutational burden of PDAC has been associated with distinct immunosuppressive mechanisms that are conditioned by the tumor-stromal environment. These defined subtypes have significance for utilizing immunotherapy in the treatment of PDAC. For example, a high concentration of lactate could weaken immune responses and lead to therapeutic resistance. Therefore, normalizing the metabolic or biologic features of the tumor microenvironment in combination with therapies targeting multiple immunosuppressive mechanism may be required to successfully implement immunotherapy in PDAC(33).

Despite intensive research on predictive biomarkers for effective responses to chemotherapy, few clinically useful markers have been identified. In most instances, sensitivity of cancer cells to drugs is likely to depend on a multiplicity of genomic and epigenomic variables. Indeed, single gene–drug associations are only rarely found(34). Thus, a panel of genes could help to monitor specific subtype treated-patients and determine biomarkers that forecast the likely course of the disease in a defined clinical population under specific treatment conditions. One of the main hallmarks of PDAC is the development of chemoresistance(35). Gemcitabine has been the mainstay of first-line PDAC treatment increasing overall survival (OS) to 5.6 months (compared with the 4.41 months OS after fluorouracil (5-FU) treatment)(36). Several targeted agents have been tested in combination with gemcitabine and have failed to confer any added benefit, with the notable exception of

erlotinib. This small molecule inhibitor of epidermal growth factor receptor (*EGFR*) tyrosine kinase conferred a very modest improvement in survival over gemcitabine alone(37). Nab-paclitaxel combined with gemcitabine was also shown to improve OS compared to gemcitabine alone(38). Subtyping PDAC has opened a door toward improved understanding of tumor biology, which would help develop and establish second and subsequent lines of targeted chemotherapies. This should challenge the current state of affairs where the majority of patients succumb within a year of diagnosis despite an increased number of therapeutic options available today.

Enrollment of PDAC patients on clinical trials is also critical for the achievement of Precision Medicine. Pancreatic Cancer Action Network (PanCan) is launching a groundbreaking initiative to dramatically improve patient outcomes and advance in the goal of doubling patients' survival by 2020. Precision Promise is the first adaptive clinical trial platform for PDAC patients. The aim of this concerted effort is to change the PDAC treatment paradigm, by testing multiple leading-edge therapeutic options at the same time, compared to the traditional approach that tests only one or two treatments. Every enrolled patient's tumor in Precision Promise will be comprehensively studied with biopsies before and after treatment (using a common platform) to obtain a better understanding about why some treatments work well in some patients and less in others and identify the corresponding molecular alterations most likely to respond to specific treatments. The data collected from the 12 organizations that are part of the consortium will be collated and analyzed together for efficient and timely dissemination of this trial data.

Improved diagnostic biomarkers for early detection and prognosis of this disease would likewise impact patient survival(39). In this regard, correlating PDAC subtypes with differences in its molecular evolution, particularly at earlier stages, could provide insight into disease mechanisms and unique serological biomarkers for each subtype. Yet finding a prognostic biomarker or panel of markers that would be able to predict patient outcomes before an initiated chemotherapeutic regimen would: (a) identify patients more likely to benefit from more aggressive therapies; (b) reduce risks of toxic side effects, and (c) encourage application of new combinations of therapies or individualized treatment protocols according to subtype-expected responses. Also, markers that display prognostic significance offer the potential to become optimal therapeutic targets in the management of PDAC(40).

Despite these advantages, there are some caveats that cannot be ignored. Although PDAC is one of the most aggressive cancers, it has a low incidence, approximately 9 new cases per 100,000 persons (0.009%)(41), which translates into lower numbers of samples. Hence, subdividing the already small patient pool will make statistical significance more difficult to achieve. Indeed, the lack of significant biomarkers may be a reflection of a very diverse patient population and hence further reduced patient pool. From a diagnostic point of view, particularly at clinical level, oncologist, pathologists, and surgeons need to be able to diagnose the disease in a defined period of time. In this regard, several questions may arise:

1. How large is the gap between genomic information and translation to clinical care?

2. How much is the real cost for diagnosing PDAC subtypes using a genomic approach?
3. Is incorporation of molecular subtyping feasible in the practice of surgery and/or in clinic?
4. Can molecular profiling integrated with histopathology improve diagnosis and patient care?
5. Can genomic and biomolecular signatures correlate to cellular phenotypes?

Addressing these issues would provide some relief to the aforementioned limitations regarding patient stratification using PDAC subtyping.

For the majority of PDAC patients (80% or more), surgery is not an option(42) as the disease is far too advanced and thus inoperable. Bailey *et al.* have approached the classification of PDAC subtypes using pancreatic tumors samples which had been surgically removed from patients (as done in previous works(4,6)). Consequently, obtaining tumor samples for all the patients (regardless of the stage of diagnosis) could represent a hindrance for the success of this classification method (it does, to some extent, also represent a biased cohort). Although these tissue-based classifications promise to drive development of new diagnostics and more tailored and effective therapies, samples obtained from biopsies lose important histological, cellular, and subcellular context that could add useful information. Continued advances in basic and clinical cancer research will likely require new comprehensive molecular profiling technologies(43) to consider information at these levels.

## Further Characterization & Future Utility

From a basic research perspective, mouse models can provide an excellent platform with which to study human disease. There are a wide variety of well-characterized and novel PDAC models available(44), but it is essential to ensure select models accurately replicate genetic alterations and overall phenotypes observed in human tumors. Being able to identify human-to-mouse disease subtype counterparts will facilitate etiological determinants, highlight the effects of mutations on various pathway activation, and improve preclinical drug testing. All three of these data points can translate into better patient care, though this will take time. Patient derived tumor xenograft (PDX) models for cancer research have attracted interest in the recent years because they retain the principal histological and genetic characteristics of their donor tumor and remain stable across passages. Thus, they represent more advanced preclinical cancer models (45). Using PDX, Rubio-Viqueira *et al*, developed a clinically meaningful *in vivo* platform for late preclinical drug development in pancreatic cancer (46). As the authors stated this model can be used as an *in vivo* screening modality to test novel drugs with therapeutic potential. Indeed, it could be a reasonable platform to evaluate markers of response, selection of appropriate drugs, and consideration of drug resistance in order to develop and apply novel technologies to treat individual patients. However, the putative loss of the tumor stroma during xenotransplantation and replacement by that of the host can affect cellular crosstalk and therefore tumor biology (47).

A more immediate impact would be subtyping all PDAC patients for improved stratification. Although these reports of PDAC subtyping have expanded our knowledge about this disease, none of the classification systems have been universally applied, which impedes clinical advancement towards Precision Medicine. Besides highlighting the different subtypes, particularly the most recent one, the true essence of this commentary is intended to go beyond mere recapitulation or marginal advancement. Improving patient survival will likely require a paradigm shift and extend well beyond enhanced understanding of this disease. Translating PDAC subtyping at the patient level will lead to identification of more effective early detection strategies, novel therapeutic targets, and advance the means to follow patient responses and outcomes. Hence, efforts should be made to build a more complete diagnostic profile for each subtype that could help in the management of this disease.

All diseases comprise an underlying complex set of alterations at a cellular or biochemical level (specific changes in concentrations and/or structure of proteins, lipids, carbohydrates, and nucleic acids) which ultimately can lead into a more aggressive phenotype. Although this DNA/RNA profile has shed light on the molecular pathology of PDAC for subtype classification, a previously validated imaging platform that would generate a biomolecular profile is likely to identify specific PDAC subtypes on a more functional level. Fourier transform infrared (FT-IR) spectroscopic imaging has become a popular technique in the field of oncology as it offers the capability of identifying a comprehensive biochemical signature of tissues and what is more interesting, of serum and other biofluids(48). It has been shown to have applicability in the diagnosis of several cancers (49–51). The loss of the histological, cellular and subcellular context is here avoided.

As stated before in Utility, Limitations and Challenges in PDAC Subtyping section, diagnosis and treatments have been made solely on histopathology for decades. Combining advances in omics technologies (genomics, transcriptomics, epigenomics, proteomics, lipidomics and metabolomics) with the strengths of PDAC subtyping could help oncologists improve disease diagnosis. As previously stated, PDAC subtype classification from surgical specimens only offers hope for less than 20% of all PDAC patients. It is critical to determine a system able to capture all PDAC subtypes regardless of disease stage, being serum the preferred source for non-invasive interrogation. Integration of all the different approaches described through this review would offer physicians the information needed for implementing Precision Medicine for PDAC patients. Genomic, transcriptomic and proteomic analyses can provide a comprehensive overview of an individual patient’s cancer, and this information can impact clinical decision making(52). Disease stratification using different technologies may establish a more clinically meaningful taxonomy of cancer and would facilitate cancer diagnosis.

This is just an example of the directions that could be taken to complement genomic subtypes with cellular and subcellular molecular fingerprints in an attempt to construct a more potent tool for PDAC patient stratification. This approach will instill new hope for improved screening and diagnosis, accurate subtyping, and more precise therapeutic intervention, which would have great impact in the treatment and management of this disease.

## Conclusion

With the projection of becoming the second leading cause of cancer-related deaths within the next ten years, PDAC remains one of the biggest challenges for the research and medical community. Subtypes described here begin to expose the biologic heterogeneity of this cancer although this variation might not be entirely captured jet by current subtyping approaches and the lack of consensus in their applicability

Despite similarities among the various PDAC subtypes, there does remain a lack of complete consensus and virtually no consideration for over 80% of the PDAC patient population. Given the potential clinical implications of employing PDAC subtyping, one emphasis should be on defining a unique and universally accepted PDAC subtyping approach. Hence, more work is needed, especially for the consideration of patients ineligible for surgery. New technologies, as DNA and RNA sequencing has done in the past decade, will have considerable impact on coalescing a broad knowledge base regarding PDAC biology. This in turn will offer new hope for future opportunities in drug development, therapeutic strategies and improved clinical outcomes. Nevertheless, this application will require modalities to translate subtype classification to the clinic in a cost-effective high-throughput manner.

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## References:

1. Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, Gingras M-C, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. Nature Publishing Group; 2016;531(7592):47–52.
2. Du Y, Zhao B, Liu Z, Ren X, Zhao W, Li Z, et al. Molecular subtyping of pancreatic cancer: Translating genomics and transcriptomics into the clinic. J Cancer. 2017;8(4):513–22. [PubMed: 28367231]
3. Pishvaian BMJ, Brody JR. Therapeutic Implications of Molecular Subtyping for Pancreatic Cancer Conventional Pathologic Subtyping of Pancreatic Neoplasms Survival Outcomes : Still Based on One-Size-Fits-All Treatment Plans. 2017;(March):1–9.
4. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011;17(4):500–3. [PubMed: 21460848]
5. Jones S, Zhang X, Parsons DW, Lin JC-H, Leary RJ, Angenendt P, et al. Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses. Science (80-) 2008;321(5897): 1801–6.
6. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SGH, Hoadley KA, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. Nature Publishing Group; 2015;47(10):1168–78.
7. Reuter JA, Spacek DV, Snyder MP. High-Throughput Sequencing Technologies. Molecular Cell. 2015 p. 586–97. [PubMed: 26000844]
8. Badea L, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepatogastroenterology. 2008;55(88):2016–27. [PubMed: 19260470]

9. Pei H, Li L, Fridley BL, Jenkins GD, Kalari KR, Lingle W, et al. FKBP51 Affects Cancer Cell Response to Chemotherapy by Negatively Regulating Akt. Cancer Cell. Elsevier Ltd; 2009;16(3): 259–66.
10. Grützmann R, Pilarsky C, Ammerpohl O, Lüttges J, Böhme A, Sipos B, et al. Gene expression profiling of microdissected pancreatic ductal carcinomas using high-density DNA microarrays. Neoplasia. 2004;6(5):611–22. [PubMed: 15548371]
11. Balagurunathan Y; Morse DL; Hostetter G; Shanmugam V; Stafford P; Shack S; Pearson J; Trissal M; Demeure MJ; Von Hoff DD; Hruby VJ; Gillies RJ; Han H. Gene expression profiling based identification of cell surface targets for developing multimeric ligands in pancreatic cancer. Mol Cancer Ther. 2008;7(9):3071–80. [PubMed: 18765825]
12. Noll EM, Eisen C, Stenzinger A, Espinet E, Muckenhuber A, Klein C, et al. CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. Nat Med. 2016;22(3):278–87. [PubMed: 26855150]
13. Waddell N, Pajic M, Patch A, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518(7540):495–501. [PubMed: 25719666]
14. Humphris JL, Patch A-M, Nones K, Bailey PJ, Johns AL, McKay S, et al. Hypermutation In Pancreatic Cancer. Gastroenterology. 2016;
15. Schlitter AM, Segler A, Steiger K, Michalski CW, Jäger C, Konukiewitz B, et al. Molecular, morphological and survival analysis of 177 resected pancreatic ductal adenocarcinomas (PDACs): Identification of prognostic subtypes. Sci Rep. 2017;7:41064. [PubMed: 28145465]
16. Raphael BJ, Hruban RH, Aguirre AJ, Moffitt RA, Yeh JJ, Stewart C, et al. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2017;32(2):185–203.e13. [PubMed: 28810144]
17. Humphrey ES, Su S-P, Nagrial AM, Hochgräfe F, Pajic M, Lehrbach GM, et al. Resolution of Novel Pancreatic Ductal Adenocarcinoma Subtypes by Global Phosphotyrosine Profiling. Mol Cell Proteomics. 2016;15(8):2671–85. [PubMed: 27259358]
18. Sturgeon CM, Duffy MJ, Hofmann BR, Lamerz R, Fritsche HA, Gaarenstroom K, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. Clin Chem. 2010;56(6):e1–48. [PubMed: 20207771]
19. Kim J, Bamlet WR, Oberg AL, Chaffee KG, Donahue G, Cao X-J, et al. Detection of early pancreatic ductal adenocarcinoma with thrombospondin-2 and CA19–9 blood markers. Sci Transl Med. 2017;9(398):eaah5583. [PubMed: 28701476]
20. Harsha HC, Kandasamy K, Ranganathan P, Rani S, Ramabadran S, Gollapudi S, et al. A compendium of potential biomarkers of pancreatic cancer. PLoS Med. 2009;6(4):2–7.
21. Pantel K, Speicher MR. The biology of circulating tumor cells. Oncogene. 2015;35:1–9. [PubMed: 25893285]
22. Pantel K, Alix-Panabières C. Real-time liquid biopsy in cancer patients: Fact or fiction? Cancer Research. 2013 p. 6384–8. [PubMed: 24145355]
23. Qi X, Yang X, Fan L, Zhang Y, Zhang F, Jiang J. Association between the spread of circulating tumor cells and breast cancer subtypes. Breast Cancer Res. 2010;12(3):402. [PubMed: 20584343]
24. Fehm T, Hoffmann O, Aktas B, Becker S, Solomayer EF, Wallwiener D, et al. Detection and characterization of circulating tumor cells in blood of primary breast cancer patients by RT-PCR and comparison to status of bone marrow disseminated cells. Breast Cancer Res. 2009;11(4):R59. [PubMed: 19664291]
25. Riethdorf S, Müller V, Loibl S, Nekljudova V, Weber K, Huober J, et al. Prognostic impact of circulating tumor cells for breast cancer patients treated in the neoadjuvant “Geparquattro” trial. Clin Cancer Res. 2017;clincanres.0255.2017.
26. Liu H, Sun B, Wang S, Liu C, Lu Y, Li D, et al. Circulating Tumor Cells as a Biomarker in Pancreatic Ductal Adenocarcinoma. Cell Physiol Biochem. 2017;42(1):373–82. [PubMed: 28558380]

27. Kulemann B, Rösch S, Seifert S, Timme S, Bronsert P, Seifert G, et al. Pancreatic cancer: Circulating Tumor Cells and Primary Tumors show Heterogeneous KRAS Mutations. Sci Rep. 2017;7(1):4510. [PubMed: 28674438]
28. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004;10(20):6897–904. [PubMed: 15501967]
29. Eason K, Sadanandam A. Molecular or metabolic reprograming: What triggers tumor subtypes? Cancer Res. 2016;76(18):5195–200. [PubMed: 27635042]
30. Daemen A, Peterson D, Sahu N, McCord R, Du X, Liu B, et al. Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors. Proc Natl Acad Sci. 2015;112(32):E4410–7. [PubMed: 26216984]
31. Samuel N, Hudson TJ. The molecular and cellular heterogeneity of pancreatic ductal adenocarcinoma. Nat Rev Gastroenterol Hepatol [Internet]. 2011;9(2):77–87.
32. He L, Long LR, Antani S, Thoma GR. Histology image analysis for carcinoma detection and grading. Comput Methods Programs Biomed. 2012;107(3):538–56. [PubMed: 22436890]
33. Knudsen E, Vail P, Balaji U, Ngo H, Botros IW, Makarov V, et al. Stratification of Pancreatic Ductal Adenocarcinoma: Combinatorial Genetic, Stromal, and Immunological Markers. Clin Cancer Res. 2017;clincanres.0162.2017.
34. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature. 2012;483(7391):570–5. [PubMed: 22460902]
35. Falasca M, Kim M, Casari I. Pancreatic cancer: Current research and future directions. Biochim Biophys Acta - Rev Cancer. Elsevier B.V; 2016;1865(2):123–32.
36. Vaccaro V, Sperduti I, Vari S, Bria E, Melisi D, Garuf C, et al. Metastatic pancreatic cancer: Is there a light at the end of the tunnel? World Journal of Gastroenterology. 2015 p. 4788–801. [PubMed: 25944992]
37. Philip PA, Mooney M, Jaffe D, Eckhardt G, Moore M, Meropol N, et al. Consensus report of the national cancer institute clinical trials planning meeting on pancreas cancer treatment. J Clin Oncol. 2009;27(33):5660–9. [PubMed: 19858397]
38. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18):1691–703. [PubMed: 24131140]
39. Agarwal B, Correa AM, Ho L. Survival in pancreatic carcinoma based on tumor size. Pancreas. 2008;36(1):e15–20.
40. Winter JM, Yeo CJ, Brody JR. Diagnostic, prognostic, and predictive biomarkers in pancreatic cancer. J Surg Oncol. 2013;107(1):15–22. [PubMed: 22729569]
41. Yeo TP, Lowenfels AB. Demographics and epidemiology of pancreatic cancer. Cancer J. 2012;18(6):477–84. [PubMed: 23187833]
42. Kleeff J, Reiser C, Hinz U, Bachmann J, Debus J, Jaeger D, et al. Surgery for recurrent pancreatic ductal adenocarcinoma. Ann Surg. 2007;245(4):566–72. [PubMed: 17414605]
43. Gerdes MJ, Sevinsky CJ, Sood A, Adak S, Bello MO, Bordwell A, et al. Highly multiplexed single-cell analysis of formalin-fixed, paraffin-embedded cancer tissue. Proc Natl Acad Sci. 2013;110(29):11982–7. [PubMed: 23818604]
44. DeCant B, Principe DR, Guerra C, di Magliano MP, Grippo PJ. Utilizing past and present mouse systems to engineer more relevant pancreatic cancer models. Front Physiol. 2014;5(Nov):1–12. [PubMed: 24478714]
45. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived Xenograft models: An emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. [PubMed: 25185190]
46. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res. 2006;12(15):4652–61. [PubMed: 16899615]
47. Behrens D, Walther W, Fichtner I. Pancreatic cancer models for translational research. Pharmacol Ther. 2017;173:146–58. [PubMed: 28174092]

48. Sahu RK, Mordechai S. Fourier transform infrared spectroscopy in cancer detection. Futur Oncol. 2005;1(5):635–47.

49. Haka AS, Volynskaya Z, Gardecki J a, Nazemi J, Shenk R, Wang N, et al. Diagnosing breast cancer using Raman spectroscopy: prospective analysis. J Biomed Opt 2009;14(5):54023.

50. Lasch P, Haensch W, Naumann D, Diem M. Imaging of colorectal adenocarcinoma using FT-IR microspectroscopy and cluster analysis. Biochim Biophys Acta - Mol Basis Dis. 2004;1688(2): 176–86.

51. Mackanos MA, Contag CH. Fiber-optic probes enable cancer detection with FTIR spectroscopy. Trends Biotechnol. Elsevier Ltd; 2010;28(6):317–23.

52. Roychowdhury S, Chinnaiyan AM. Translating cancer genomes and transcriptomes for precision oncology. CA Cancer J Clin. 2016;66(1):75–88. [PubMed: 26528881]

**Key Messages:**

- Pancreatic cancer can no longer be considered as one disease.
- The heterogeneity underlying pancreatic cancer patients makes therapeutic options based on one-size-fits-all approach ineffective.
- Identifying patients that could benefit from a specific treatment would help to avoid futile therapy approaches and to improve outcomes and quality of life of those whose long-term survival is unpromising.

**Table 1:**

Comparison of the distinct molecular subtypes of Pancreatic Cancer

| Ref | Classification | Survival (months) | Source | Methodology | Comments |
|---|---|---|---|---|---|
| **Jones** (5) | ***12*** *signaling pathways* | Not specified | 24 human PDAC tissue | Sequencing of protein-coding genes | First reference to targeted therapies. Target physiologic effects of the altered pathways/ processes rather than individual genes. |
| **Collison** (4) | **Classical:** *adhesion-associated and epithelial genes.* | 23 | 27 Microdissected human PDAC tissue 19 Human PDAC cell lines 15 Mouse PDAC cell lines | Global gene expression analysis | Only two first subtypes inhuman PDAC cell lines. **QM**-PDA subtype lines gemcitabine sensitive. **Classical** subtype cell lines Erlotinib sensitive. |
| | **Quasimesenchymal** (QM): *mesenchyme associated genes.* | 6,6 | | | |
| | **Exocrine-like**: *tumor cell derived digestive enzyme gene.* | 19.7 | | | |
| **Moffitt** (6) | **Stroma-specific subtypes:** | | 145 primary & 61 metastatic PDAC tumors 17 PDAC cell lines 46 pancreas & 88 distant site adjacent normal samples | Global gene expression analysis. Validation with RNA sequencing | Collisson's "**classical**" subtype overlapped with Moffitt's **classical** subtype. **QM** subtype appeared to be a mixed collection of genes from "**basal-like**" and stromal subtypes. All cell lines were classified as **basal-like**. |
| | **Normal**: *markers for pancreatic stellate cells.* | 24 | | | |
| | **Activated**: *genes associated with macrophage, genes with role in tumor promotion.* | 15 | | | |
| | **Tumor-specific subtypes:** | | | | |
| | **Classical**: *high adhesion-associated, ribosomal and epithelial gene expression, and elevated GATA6 expression.* | 19 | | | |
| | **Basal-like**: *laminins and keratins.* | 11 | | | |
| **Waddel** (13) | **Subgroups of PDAC based on the frequency and distribution of structural rearrangements:** | | 100 PDAC samples | Whole-genome sequencing and copy number variation analysis | Define putative biomarkers of therapeutic responsiveness for platinum-based chemotherapy. |
| | **Stable**: *< 50 structural variation events and often exhibited widespread aneuploidy suggesting defects in cell cycle/mitosis.* | Not specified | | | |
| | **Locally rearranged**: *significant focal event on one or two chromosomes. Common focal amplifications in KRAS, SOX9 and GATA6 and therapeutic targets such as ERBB2, MET, CDK6, PIK3CA and PIK3R3.* | Not specified | | | |
| | **Scattered**: *moderate range of non-random chromosomal damage* | Not specified | | | |

| Ref | Classification | Survival (months) | Source | Methodology | Comments |
|---|---|---|---|---|---|
| | *and less than 200 structural variation events* | | | | |
| | **Unstable**: *Large number of structural variation events (<200; maximum of 558). Defects in DNA maintenance.* | 35 platinum-based chemo | | | |
| | | | | | |
| | | | | | |
| **Bailey** (1) | **Squamous**: *enriched for activated α6β1 anc α6β4 integrin signaling and activated EGF signaling. Hypermethylation of genes that govern pancreatic endodermal cell-fate determination leading to a complete loss of endodermal identity.* | 13.3 | 456 primary tumors 41 patient-derived cell lines Mouse PDAC cell lines | Integrated genomic analysis (whole-genome and deep-exome sequencing, with gene copy number analysis); RNASeq | Very similar to Collison except for the **Immunogenic**. Overlapping: **Squamous**, **QM** and "**basal-like**"; **pancreatic progenitor** and **classical**; **ADEX** and **exocrine-like**. |
| | **Aberrantly differentiated endocrine exocrine (ADEX)**: *transcriptional networks in later stages of pancreatic development and differentiation. Transcription factors NR5A2, MIST1 important in acinar cel. differentiation and pancreatitiss regeneration. Genes associated with endocrine differentiation and MODY.* | 25.6 | | | |
| | **Pancreatic progenitor**: *pancreas development, Gene programs regulating fatty acid oxidation, steroid hormone biosynthesis, drug metabolism and O-linkec glycosylation of mucins.* | 23.7 | | | |
| | **Immunogenic**: *Immune infiltrate. B cell signaling pathways, antigen presentation, CD4+ T cell, CD8+ T cell and Toll-like receptor signaling pathways* | 30 | | | |
| | | | | | |
| **Raphael** (16) | **Basal-like/squamous**: *enriched in TP53 mutations. Specific miRNA and DNA methylation patter.* | Not specified | 150 pancreatic cancers | Multi-platform analysis: genomic, transcriptomic, and proteomic profiling | Recurrent mutations identified *(KRAS, TP53, CDKN2A, SMAD4, RNF43, ARID1A, TGFbR2, GNAS, PBRM1)* common to other subtypes. |
| | **Classical/pancreatic progenitor**: *enriched in GNAS mutations. Elevated RTK and MTORC signaling* | Improved OS | | | |